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New records of *Melampsora* species on willows in China

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Abstract — Three rust species, *Melampsora chelidonii-pierotii*, *M. humilis*, and *M. kiusiana,* are recorded for the first time in China based on morphological features and rDNA ITS1-5.8S-ITS2 sequence information. New hosts are also reported: *Salix raddeana* for *M. chelidonii-pierotii*; *S. caprea* and *S. siuzevii* for *M. humilis*; and *S. triandra* and *S. starkeana* for *M. kiusiana*. The morphology, host range, and distribution of these species in China are provided.
Key words — *Melampsoraceae*, *Salicaceae*, taxonomy

Introduction

The genus *Melampsora* Castagne (*Melampsoraceae*, *Pucciniales*) contains about 90 species (Kirk et al. 2008). The species are characterized by formation of crust-like telia comprising sessile, laterally adherent single-celled teliospores under the host epidermis (Pei et al. 2005). *Melampsora* species cause serious damage to cultivated willows, which are a significant resource for renewable biomass production (Royle et al. 1992). In China, 25 *Melampsora* species have been recorded on willows (Farr & Rossman 2011), of which 24 species were reported on ~72 cultivars representing 18 sections in *Salix*, while one species, *M. kamikotica*, was reported on *Chosenia* (Liu 2005, Tai 1979, Wang et al. 1980, Zhuang 1986, 1989, 1994, Zhuang & Wang 2006, Zhuang & Wei 2002, 2003). However, the taxonomic identities of these reported species need reevaluation due to taxonomic confusion and morphological similarity among species.

Melampsora species on willows deposited in several herbaria in China were investigated by morphological examinations and rDNA ITS1-5.8S-ITS2 sequence analyses. Three species — *M. chelidonii-pierotii*, *M. humilis*, and *M. kiusiana* — were represent new records for China. Their morphology, host plants, and geographical distribution in China are described in the present paper.

Materials & methods

Herbarium specimens used for morphological examinations were borrowed from the Mycological Herbarium of Institute of Microbiology, Chinese Academy of Sciences (HMAS); Inner Mongolia Agricultural University (HNMAP); the Mycological Herbarium of College of Forestry, Northwest A & F University, China (HMNWFC); Systematic Mycology and Microbiology Laboratory, Agricultural Research Service, USDA, USA (BPI); and the Mycological Herbarium of the Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Japan (TSH).

The collections were examined morphologically using light microscope (LM) and scanning electron microscopy (SEM). For LM examination, the urediniospores or thin sections of uredinia and telia were mounted in a drop of lactophenol solution on a microscopic slide. Germ pores of urediniospores were observed by the method of Liang (2006). For SEM, urediniospores or sori obtained from specimens were attached to specimen holders by double-sided adhesive tape and coated with platinum-palladium with an E-1030 Ion Sputter Coater (Hitachi, Tokyo, Japan). They were examined using an S-4200 SEM (Hitachi, Tokyo, Japan) operating at 15 kV.

DNA was extracted from specimens by the methods of Tian et al. (2004) and fragments of rDNA ITS1-5.8S-ITS2 were amplified by polymerase chain reaction (PCR) techniques. Amplified PCR products were directly sequenced, and obtained sequences were compared with data deposited in GenBank using BLAST to calculate similarity values.

Taxonomy

Melampsora chelidonii-pierotii Tak. Matsumoto, Bot. Mag. (Tokyo) 40: 46, 1926
Fig. 1

Spermogonia and Aecia not seen in Chinese specimens. Uredinia mostly hypophyllous, 0.4–0.8 mm; Urediniospores obovoid, ellipsoid or broadly ellipsoid, 16–26 × 11–18 µm, wall 1.5–2.6 µm thick, echinulate, germ pores 3–6, bizonate or tending to be scattered; Paraphyses mostly pear- or lollipop-shaped and interspersed throughout uredinia, evenly thickened at apex or slightly thickened at apex, up to 7 µm. Telia amphigenous or epiphyllous, 0.2–0.4 mm, subcuticular; Teliospores 12–41 × 5–14 µm, wall 1.6 µm thick, not thickened at apex.

Specimens examined: **China**, **Shaanxi Province**, Shang Bai Yun, Tai Bai Shan, II, III on *Salix chaenomeloides* Kimura, 4 September 1985, Cheng-Ming Tian (HMNWFC13); **Jilin Province**, Antu county, II, III on *S. raddeana*

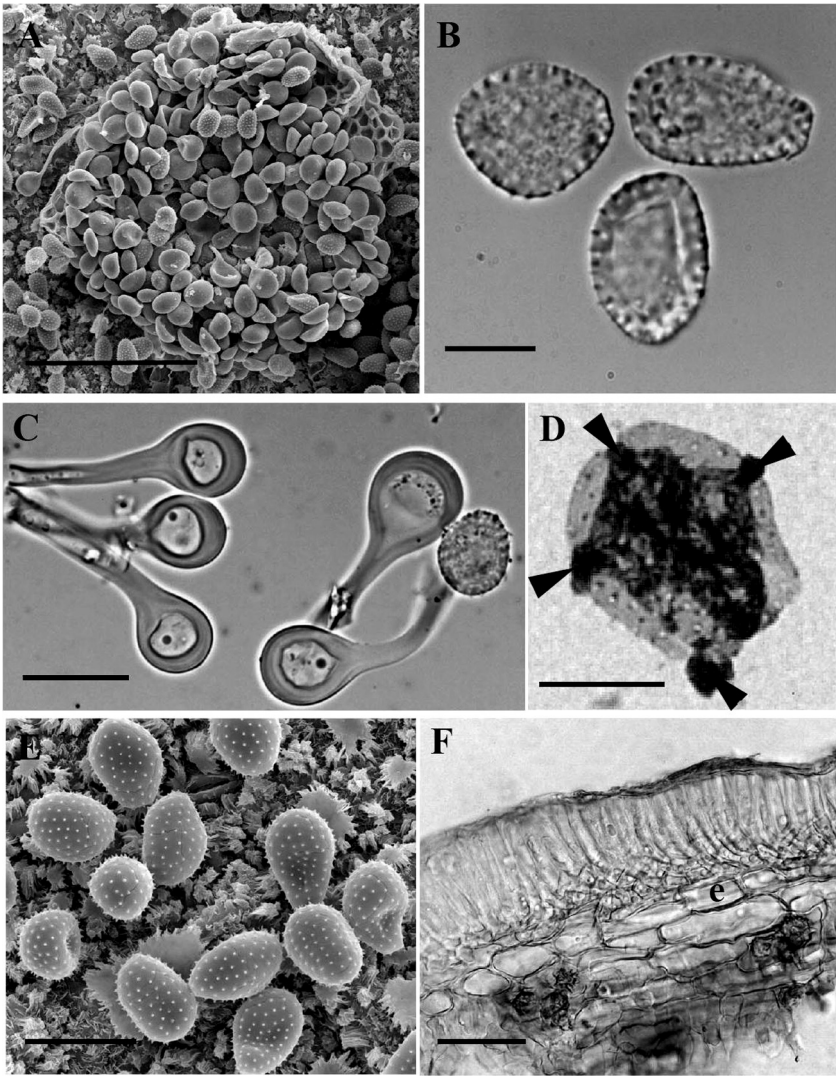

Figure 1. *Melampsora chelidonii-pierotii* (HMNWFC13). Morphology — A: Uredinium observed by SEM. B: Obovoid or ellipsoid urediniospores observed by LM. C: Paraphyses with evenly thickened apex or slightly thickened apex. D: Scattered germ pores (arrows). E: Echinulate urediniospores observed by SEM. F: Subcuticular telium (e = epidermal cells of the host). Scale bars: A = 100 μm; B, D = 20 μm; C = 10 μm; E = 15 μm.

Laksch. ex Nasarow, 16 July 1960, Yu-Chuan Yang, (BPI1109442); **Anhui Province**, Qingyang County, II, III on *Salix* sp., 9 November 1932, Cheo S.Y. (BPI023209, BPI023210).

Comments: Matsumoto (1926) described *M. chelidonii-pierotii* based on a rust fungus on *S. pierotii* from Honshu, Japan. This species is characterized by subcuticular telia and mostly obovoid or ellipsoid urediniospores. Based on the morphology, one Chinese specimen on *S. chaenomeloides* from Shaanxi Province, which had previously been identified as *M. coleosporioides* (Cao & Li 1999), was redetermined to *M. chelidonii-pierotii,* as were three other specimens from China labeled as *M. larici-epitea* (one on *S. raddeana* and two on an unidentified *Salix* sp.). These specimens possessed subcuticular telia, and urediniospore dimensions agreed well with the descriptions of *M. chelidonii-pierotii* (Hiratsuka & Kaneko 1982). Two rDNA ITS1-5.8S-ITS2 sequences obtained from specimens BPI1109442 and BPI023210 also supported the morphological identification. A BLAST search of the GenBank database showed a 100% similarity of these sequences with *M. chelidonii-pierotii* strain E-1g (AB646769.1, deposited by Yamaoka et al. 2010). The teliospores of the Chinese specimens were smaller (12–41 × 5–14 µm) than in the Japanese specimens (18–60 × 14–23 µm, Hiratsuka & Kaneko 1982). However, the teliospore position separated these specimens from the morphologically similar species, *M. coleosporioides*.

Melampsora chelidonii-pierotii has been reported on *Salix pierotii* and *S. chaenomeloides* from Japan and Russia (Hiratsuka & Kaneko 1982; Azbukina 1974). Here we report *S. raddeana* as a new host. Although this rust species was identified on willows from Shaanxi, Jilin, and Anhui Provinces in China, a wide distribution has been expected throughout China based on the wide distribution of *S. chaenomeloides* in the northeast, northwest, and central regions the country (Zheng 1983). Japanese mycologists (Matsumoto 1926, Kaneko & Hiratsuka 1981) reported its aecial stage on *Chelidonium majus* var. *asiaticum* and *Corydalis incisa* and demonstrated experimentally the heteroecious life cycle between *S. chaenomeloides* and *Corydalis incisa.* However, host alternation of *M. chelidonii-pierotii* in China is still unknown.

Melampsora humilis Dietel, Bot. Jahrb. 32: 50, 1902 Fig. 2

Spermogonia and Aecia not seen in Chinese specimens. Uredinia mainly hypophyllous, 0.1–0.4 mm; Urediniospores globoid or broadly ellipsoid, 14–22 × 11–19 µm, wall 1.5–2.8 µm thick, echinulate, germ pores 3–7, scattered; Paraphyses mostly pear- or lollipop-shaped and peripheral or interspersed throughout uredinia, evenly thickened or slightly thickened at apex, up to 4.6 µm. Telia mainly hypophyllous, 0.1–0.4 mm, subepidermal; Teliospores 11–36 × 5–14 µm, wall sometimes slightly thickened at apex, 1.5–3.2 µm.

Specimens examined: **CHINA**, **Hebei Province**, Xiao Wu Tai Shan, II, III on *S. caprea* L., 22 September 1990, Jian-Yun Zhuang (HMAS67392); **Inner Mongolia**, Genhe City, II, III on *S. siuzevii* Seemen, 19 August 1993, Zhen-Shi Hou (HNMAP1594. HNMAP3197).

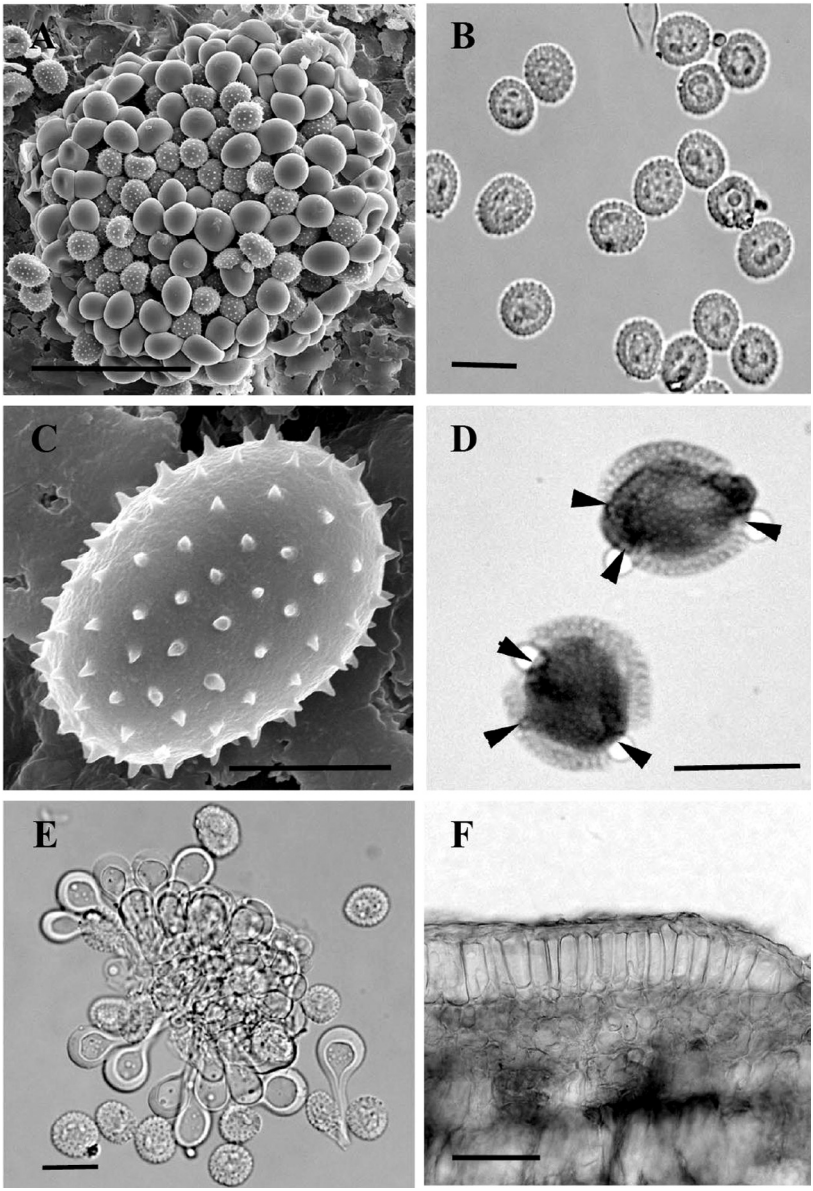

Figure 2. *Melampsora humilis* (HNMAP1594). Morphology — A: Uredinium with peripheral paraphyses. B: Globoid or broadly ellipsoid urediniospores observed by LM. C: Echinulate urediniospore observed by SEM. D: Scattered germ pores (arrows). E: Paraphyses slightly thickened at apex. F: Subepidermal telium with teliospores slightly thickened at apex. Scale bars: A = 50 μm; B = 20 μm; C = 6 μm; D, E = 15μm; F = 25 μm.

Comments: Dietel (1902) proposed *M. humilis* based on a rust fungus on *S. koriyanagi* in Japan. Morphologically, this species is closely related to *M. epitea* but differs from the latter by its shorter teliospores (Hiratsuka & Kaneko 1982). A Chinese rust specimen on *S. caprea*, collected from Hebei Province and previously identified as *M. larici-capraearum* (Tai 1979), was redetermined to *M. humilis*. Two rust specimens on *S. siuzevii* from Inner Mongolia, previously identified as *M. repentis* in China (Liu 2005), also represents *M. humilis*. Although the urediniospore dimensions and shape in these Chinese specimens agree with *M. epitea* as reported in Japan (Hiratsuka & Kaneko 1982), the teliospores are shorter (<36 µm) than in *M. epitea* (<50 µm). Sequence comparison also supported separation of the rust from *M. epitea*. The rDNA ITS1-5.8S-ITS2 sequences obtained from two specimens, HMAS67392 and HNMAP1594, showed a 99% similarity with sequences from *M. humilis* (TSH-R10561 and TSH-R10220) from *S. integra* in Japan (unpublished). The data were distinct from sequences of two formae specials of *M. epitea* (AY444777.2 and AY444778.2; 98% similarity) deposited by Pei et al. (2005).

Two willow hosts, *S. integra* and *S. koriyanagi*, were reported as hosts of *M. humilis* from Sakhalin, Taiwan, and Japan (Hiratsuka & Kaneko 1982). Here we report *M. humilis* for the first time for mainland China and *S. caprea* and *S. siuzevii* as new hosts. The aecial host and life cycle information of *M. humilis* have not been reported yet, and host alternation is also unknown for China.

Melampsora kiusiana Hirats. f., Bot. Mag. (Tokyo) 57: 281, 1943 Fig. 3

Spermogonia and Aecia not seen in Chinese specimens. Uredinia hypophyllous, 0.1–0.4 mm; Urediniospores globoid or broadly ellipsoid, 9–18 × 6–14 µm, wall 1.5–2.1 µm thick, echinulate, germ pore scattered or tending to bizonate, 2–5; Paraphysis pear- or lollipop-shaped and interspersed throughout uredinia, wall thickened at apex, up to 8 µm. Telia amphigenous, 0.1–0.4 mm, subepidermal; Teliospores 14–56 × 5–12 µm, wall 1 µm thick, slightly thickened at apex.

Specimens examined: **China**, **Inner Mongolia**, Gen He City, II, III on *S. triandra* L., 23 August 2005, Zhen-Shi Hou (HNMAP3161, HNMAP3181); Oroqen Qi, II, III on *S. starkeana* Willd., 23 August 1995, Zhen-Shi Hou (HNMAP1972).

Comments: Hiratsuka (1943), who described *M. kiusiana* on *S. subopposita* in Kyushu, Japan, characterized it with small urediniospores and slender teliospores. We redetermined two rust specimens on *S. triandra* from Inner Mongolia, previously reported as *M. repentis*, as *M. kiusiana*. A rust specimen on *S. starkeana*, identified as *M. lapponum* (Liu 2005), also resembled *M. kiusiana*. Morphological examinations revealed that the rusts specimens from *S. triandra* and *S. starkeana* had smaller urediniospores (9–18 × 6–14 µm) than other morphologically similar species, *M. epitea* (13–23 × 11–18 µm) and

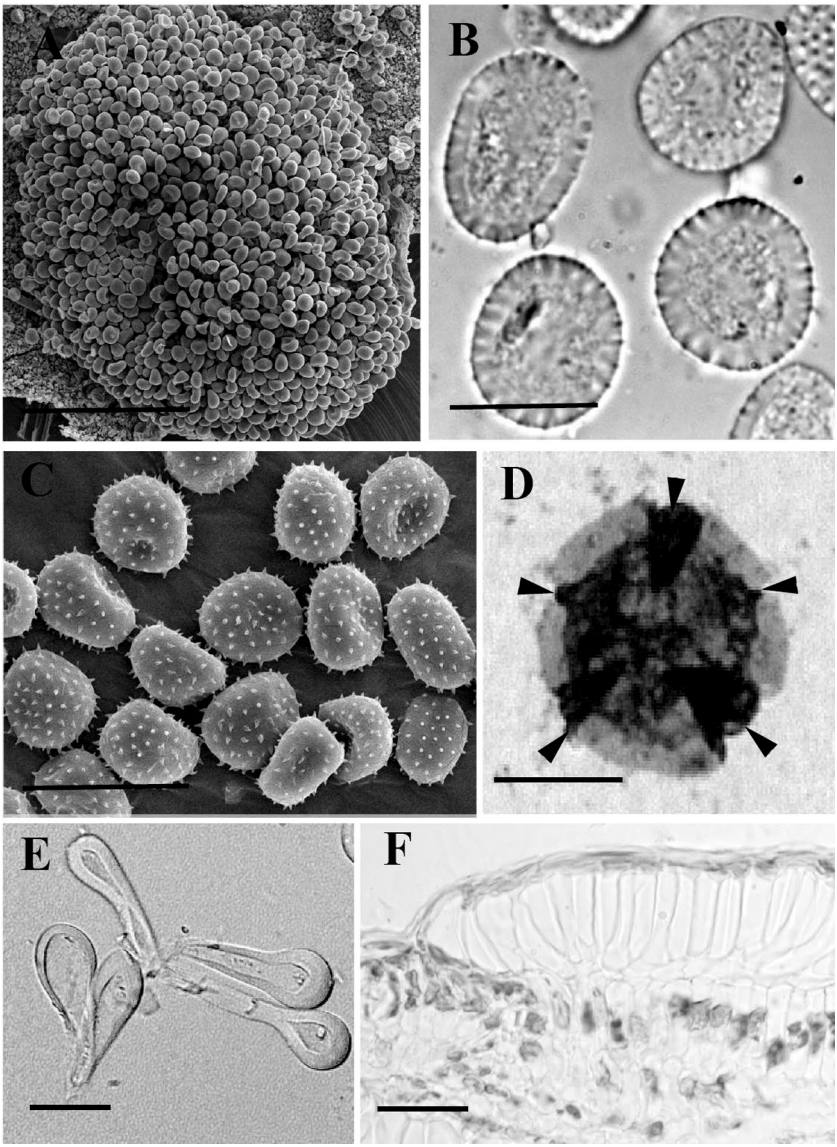

Figure 3. *Melampsora kiusiana* (HNMAP3181). Morphology — A: Uredinium with abundant paraphyses. B: Globoid or broadly ellipsoid urediniospores observed by LM. C: Echinulate urediniospores observed by SEM. D: Scattered germ pores (arrows). E: Paraphyses with evenly thickened or slightly thickened apex. F: Subepidermal telium with slender teliospores. Scale bars: A = 150 μm; B = 10 μm; C, E = 20 μm; D = 5 μm; F = 25 μm.

M. humilis (16–20 × 13–18 µm) (Hiratsuka & Kaneko 1982). The urediniospores of these specimens were also smaller than *M. repentis* (13–17 × 12–14 µm) and *M. lapponum* (20–21 × 15–16 µm) (Klebahn 1914). The teliospores were slightly larger than those of *M. epitea* (25–50 × 8–14 µm), *M. humilis* (13–33 × 6.5–15 µm), *M. repentis* (16–48 × 7–14 µm), and *M. lapponum* (30–50 × 6–12 µm) (Hiratsuka & Kaneko 1982, Klebahn 1914). Rusts on *S. triandra* and *S. starkeana* agreed with the morphological description of *M. kiusiana* (Hiratsuka & Kaneko 1982). Sequences from these specimens also differed from *M. epitea* and *M. humilis*. The rDNA ITS1-5.8S-ITS2 sequences obtained from HNMAP3161, HNMAP3181, and HNMAP1972 were somewhat distinct from *M. epitea* f. *laricis-epitea* (AY444778.2; 98% similarity) deposited by Pei et al. (2005). The sequences were also distinct from *M. humilis* (98% similarity) obtained from *S. caprea* and *S. siuzevii* in China. Morphological and molecular comparisons confirmed these Chinese specimens as *M. kiusiana*, and this represents the first report of *M. kiusiana* from Inner Mongolia in China. In Japan, *S. subopposita* was reported as a host of this species (Hiratsuka 1943), and the two willow species, *S. triandra* and *S. starkeana*, are new hosts of *M. kiusiana.* An aecial host of this species has not yet been reported for *M. kiusiana,* and life cycle information is also unknown for China.

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